

REPRODUCTIVE ECOLOGY OF TWO SPECIES OF *PHOTINUS* FIREFLIES (COLEOPTERA: LAMPYRIDAE)*

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INTRODUCTION

The elaborate flash communication system of lampyrid beetles in the genus *Photinus* has been well-documented (Lloyd 1966; Buck and Buck 1972; Carlson and Copeland 1988), but little is known concerning details of the mating systems or reproductive behavior for most members of this group (but see Cicero 1983; Wing 1984, 1985). *Photinus collustrans* is a Florida grassland species with brachypterous females that mate only once (Wing 1984). This study reports the mating behavior of two grassland *Photinus* species in Massachusetts, which in contrast have relatively long-lived females that mate repeatedly throughout their adult lives. In addition, this study documents a marked seasonal shift within *Photinus* populations in the operational sex ratio, defined as the ratio of breeding males to females (Emlen and Oring 1977). A shift from highly male-biased sex ratios at the beginning of the flight season to female-biased ratios later in the season appears to be associated with changes in the mating behavior of the two *Photinus* species.

METHODS

Studies of *Photinus aquilonius* Lloyd and *P. marginellus* LeConte were conducted in 1986–87 and 1989 at the Case Estates of Harvard University in Weston, Massachusetts. *P. aquilonius* was active from late June to late July in an open field bordered by woodlands, and *P. marginellus* was active from early July to late August in an area of mowed grass under a grove of cherry trees. In both species, males take flight shortly after sunset and emit single flashes at irregular intervals (at 70°F, 5–7 sec intervals for *P. aquilonius*, and 3–4 sec intervals for *P. marginellus*). Although

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females of both species are capable of flight, they generally remain stationary on perches in the vegetation for the duration of each nightly mating period. Females respond to conspecific males with single flash responses given at a species-specific time delay following the male flash (at 62°F, female response latency for *P. aquilonius* is approximately 2 sec, and < 1 sec for *P. marginellus*). Species identifications were kindly provided by Dr. James E. Lloyd.

Operational Sex Ratio

Two measures of operational sex ratio were used in this study. We measured population-wide sex ratios of breeding individuals by separately censusing signalling males and responding females. Since only those beetles actively attempting to find a mate will flash, this flash census method provides a measure of breeding sex ratio. In both *Photinus* species male density was estimated several times nightly by enumerating male flashes in a known area over a 2 min time interval. For *P. aquilonius*, male density was estimated by counting the number of male flashes that occurred within 1 m while an observer walked along a 75 m transect through the center of male activity. Flashes from flying males as well as males on the ground were counted on hand tally counters. Because *P. marginellus* males flew within a much more restricted area, male density was estimated by a stationary observer counting male flashes occurring over a 2 min period in a 9 m × 3 m area comprising the main center of male activity. For both species, females were found by their flashed responses to either male flashes or artificial male flashes simulated by a penlight. Female locations were marked with survey flags. Standardized female densities are reported here, calculated as the number of females found on a given night divided by total time spent searching for females.

A second measure of operational sex ratio was the number of signalling males observed within a fixed radius of each responding female (50 cm radius for *P. aquilonius*, 20 cm for *P. marginellus*). This is referred to as the local sex ratio, and was estimated several times through the nightly mating period for each focal female (see below).

Mating Behavior

Males and females were individually marked by elytral dots using Unipaint PX-21 oil-based paint markers (Faber Castell), and elytral lengths were measured to the nearest 0.1 mm using dial calipers.

Females were randomly selected for 5 min focal observation periods, during which a number of behaviors were recorded: 1) height off the ground of each female's perch, 2) local sex ratio (number of signalling males within a fixed radius of the female), 3) number of flashes given by each signalling male and the number of female responses to that particular male. Female response rates were calculated as the proportion of male flashes to which a female responded. In addition, the durations of two distinct stages of copulation were measured. After contacting a female, the male mounted the female dorsally and attempted to insert his aedeagus into her genital opening. This dorsal mount phase was defined as stage I, and was usually accompanied by repeated insertion and removal of the male's aedeagus from the female's genital opening. The beginning of the second stage of copulation was defined when the male swivelled around 180 degrees to face away from the female, the pair assuming a tail-to-tail position and remaining tightly coupled through their genitalia. Pairs in stage II were checked every 20 min to determine the duration of the second stage of copulation.

RESULTS

Photinus aquilonius: Mating Behavior

Male *P. aquilonius* began flying approximately 20 min after sunset, and the male flight period lasted about 1 h. Males generally flew within 2 m of the ground. Females responded with single flashes from perches on grass or small shrubs (mean perch height \pm SE = 27 ± 3 cm, $n = 85$). Females often curled their abdomens toward the direction of the male's flash when responding. After an inspection dialogue of variable length, males landed within 50 cm of the female, and continued the final approach by walking to the female's perch.

The first stage of copulation, during which the male was mounted dorsally on the female, was quite variable in duration, ranging from 1 to 53 min (median = 5 min, $n = 23$). In most

instances (14 out of 17 dorsal mounts observed in their entirety), this dorsal mount stage was accompanied by repeated insertion and withdrawal of the male's aedeagus from the female's genital opening (10–20 times per min). In several cases, a droplet of liquid was observed on the tip of the male's aedeagus after withdrawal from the female; males wiped this droplet off by drawing the tip of the aedeagus across the edge of the female's elytra or across the edge of a leaf. After the male swivelled around to face in the opposite direction at the beginning of stage II, the pair remained immobile for the remainder of copulation. The second stage of copulation ranged from 1.25 to 8.5 h (median = 7.5 h, $n = 13$), most often lasting all night until dawn.

Females were responsive to both male flashes and simulated male flashes over several nights. Individually marked females were observed mating on several successive nights. Females appeared to respond differentially to particular males, as measured by the percentage of female responses to each male's flash signals. Males that eventually mated with females on average elicited a female response rate of 44.3% (± 6.4 , $n = 12$). Other males that dialogued with these same females but failed to mate elicited significantly lower response rates (mean female response to non-mating males = 19.0% ± 5.2 , $n = 22$; Mann-Whitney U test, $p = 0.0006$).

Male *P. aquilonius* were significantly larger than females (mean elytral length for males = 7.4 ± 0.2 mm, for females = 6.3 ± 0.1 mm: t-test, 46 df, $p < 0.0001$). Female abdomens were often noticeably distended with eggs, and one early-season gravid female (elytral length 6.8 mm) was dissected and found to have 51 mature eggs.

During the 1989 flight season, 14 out of 26 individually marked females were resighted at least once, with the time elapsed from initial marking to last sighting ranging from 1 to 21 days: three females were resighted at least 10 days after being marked. In contrast, only two out of 12 marked males were resighted, at 2 and 4 days after being marked. Elytral color on those marked individuals that were followed through time gradually turned from black to yellowish grey, and elytral color appears to serve as a rough indicator of age within a species.

Throughout the flight season, orb-weaving spiders in the genus *Araneus* appeared to be a major source of mortality for *P.*

aquilonius males. Dead males were frequent prey in spider webs, and often two or more males were observed wrapped up in the same web. Several observations were made of wrapped, and presumably dead, firefly males responding to nearby male flashes or to simulated flashes with a female-like flash pattern. These dead-male responses only occurred when the resident spider was in direct contact with its prey; other males trapped in the same webs sometimes glowed continuously or flashed sporadically, but never flashed in response.

Photinus marginellus: Mating Behavior

The male flight period for *P. marginellus* began 10 min after sunset, and continued for approximately 40 min. The mating arena was restricted to a ~100 m² area beneath a canopy of cherry trees, and males generally flew less than 1 m from the ground. Female perches were mostly located in mown grass, although some females were found in taller grass at the lawn margin. As a result, *P. marginellus* perch height was lower than that observed for *P. aquilonius* (mean = 9 ± 1 cm, $n = 80$). Toward the end of the flight season in mid-August, however, *P. marginellus* females appeared to move to higher perches, and were consistently found up in the tree canopy at 3–7 m.

Signalling males landed after a few female responses, but were observed to take flight again if they could not locate the female within several minutes. Females frequently left their perches if they had not found mates by the end of the nightly male flight period, and late in the season females that were unsuccessful in attracting a mate often flew several meters to new perches. The first stage of copulation was generally shorter than in *P. aquilonius*, ranging from 17 sec to 25 min (median = 6.5 sec, $n = 63$), and was characterized by the same male genital insertion-removal behavior that was observed in *P. aquilonius*. At the beginning of stage II, females often moved from their original perch, dragging the coupled male behind them. The second stage of copulation was also shorter in duration, ranging from 0 to 188 min (median = 91.5 min, $n = 66$). Most copulating *P. marginellus* pairs had separated by midnight, and with a single exception females did not remate on the same night. One instance was observed of a pair

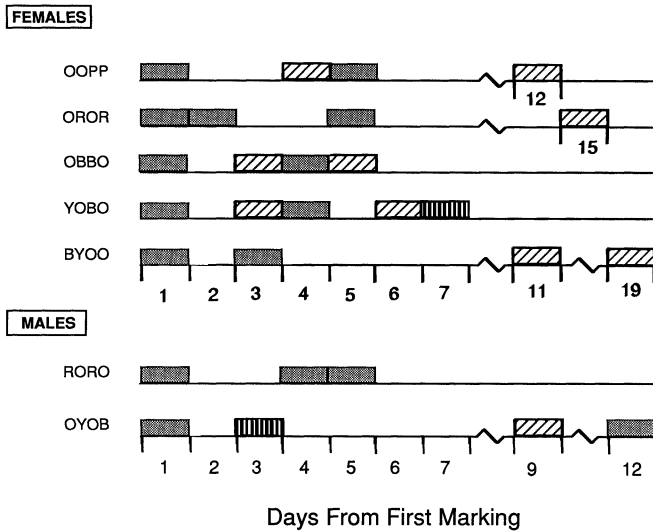


Figure 1: Mating histories for representative marked *Photinus marginellus* females and males (four-letter codes are individual identification markings). Grey rectangles indicate nights on which individuals were observed mating. Diagonally hatched rectangles indicate nights on which beetles were flashing (males signalling or females responding) but were not mating by the end of the nightly flight period. Rectangles with vertical bars indicate repeated stage I copulation, without proceeding to stage II.

separating after stage I and the female remating 1 h later with a different male.

As in *P. aquilonius*, observations of focal females indicated that females responded differentially to particular males. Those males that eventually succeeded in mating with a female elicited a significantly higher female response rate ($49.6\% \pm 4.1$, $n = 36$ males), than did other signalling males that did not end up mating with these females ($21.7\% \pm 5.3$, $n = 30$ males: Mann-Whitney U test, $p < 0.0001$).

Both males and females were found to mate repeatedly over the course of several nights, and in one instance the same male-female pair was observed mating on two consecutive nights. Representative mating histories for some marked *P. marginellus* females and males are shown in Figure 1.

In *P. marginellus*, male elytral length (mean = 5.7 mm \pm 0.1, n = 36) was not significantly greater than female elytral length (mean = 5.6 mm \pm 0.1, n = 72: t-test, p = 0.69). The abdomens of gravid females often protruded considerably beyond the elytral tips, and one late-season female (elytral length 5.9 mm) collected 19 days after initial marking still contained 18 mature eggs. During the 1989 flight season, 21 out of 72 marked females were resighted at least once with a mean time from initial marking to last sighting of 11 days (range 1–41 days). Seven out of 33 marked males were resighted, with a mean time to resighting of 4 days (range 1–11 days). The mean time from initial marking to last sighting was significantly greater for females than for males (t-test, p = 0.04). As in *P. aquilonius*, *P. marginellus* males were frequently found in spider webs, including one male that had been marked the previous night and was trapped in a web as he engaged in dialogue with a nearby female.

Operational Sex Ratios

For both *Photinus* species studied, operational sex ratios showed a marked shift from male-biased early in the flight season to female-biased later in the flight season (Figure 2). Male density reached a peak early in the flight season for both species, and then showed a fairly monotonic decline throughout the rest of the season. In contrast, female density remained relatively constant, with some fluctuation through the season. By the last two weeks of the flight season male density was quite low yet many responsive, and apparently gravid, females remained actively searching for mates.

Local sex ratios, measured as the number of signalling males within a fixed radius of a responding female, also showed a similar trend through the flight season (Figure 3). Early in the flight season of both species, there were two to three dialoging males per female; by mid-season there was approximately one male per female, while for the latter half of the season, there was less than one male per female. The 1989 flight season of *P. marginellus* was divided into early-season (days 1–17), mid-season (days 18–28), and late-season (days 29–49). These three time periods were characterized by a significant decline in the numbers of actively searching males per responsive female: mean local sex ratio early in the season was 1.7 males per female (\pm 0.2, n = 63), compared

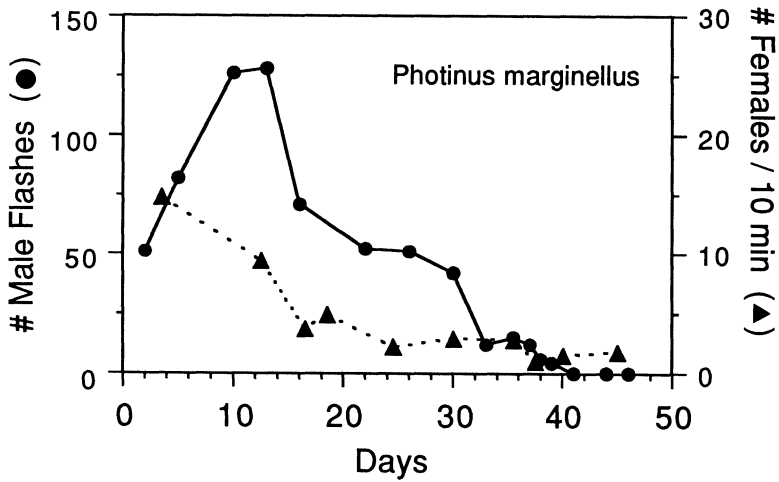
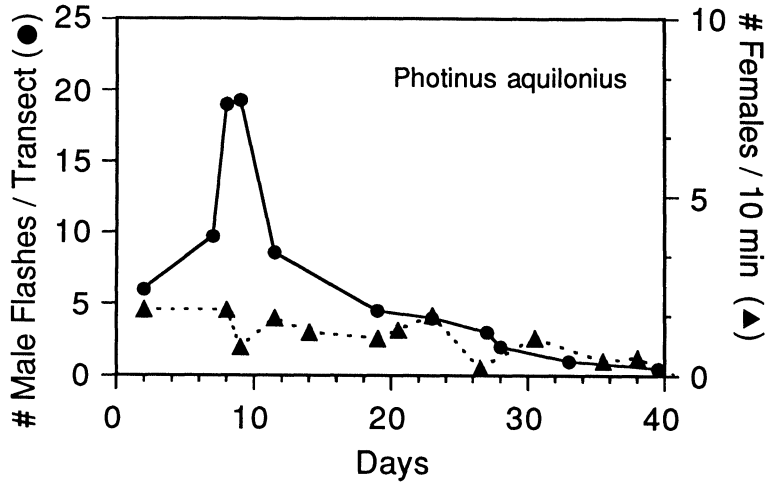


Figure 2: Operational sex ratio estimated by flash censuses of males (●) and females (▲) over the 1989 flight season for *Photinus aquilonius* and *Photinus marginellus*. Counts shown are means for censuses conducted over two successive nights.

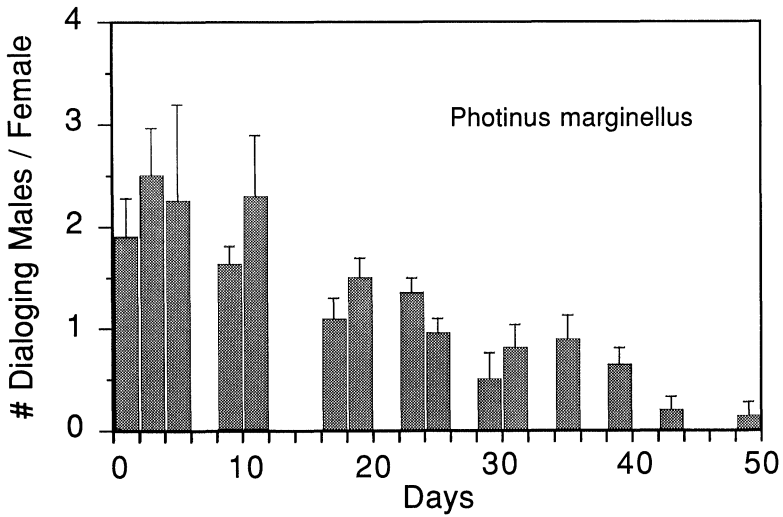
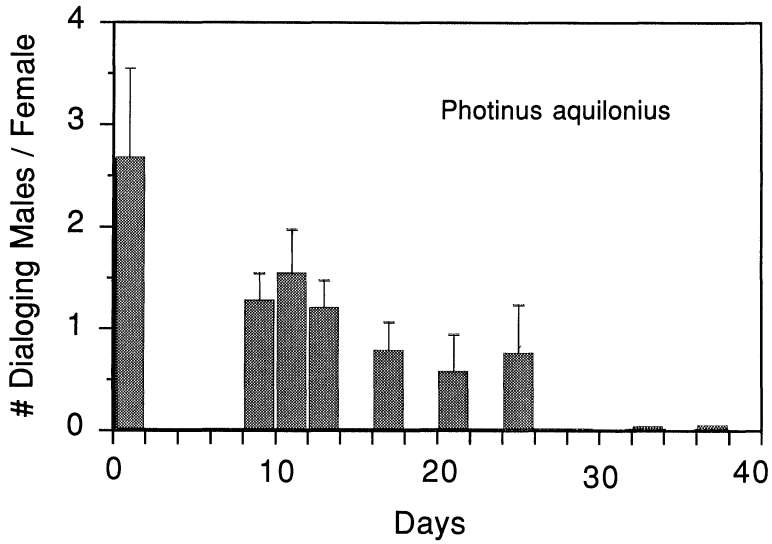


Figure 3: Local sex ratio as the maximum number of signalling males found within a fixed radius of each female (50 cm for *P. aquilonius*, 20 cm for *P. marginellus*). Means (+ SE) are shown for all females observed over two successive nights (n ranges from 7–34 females for each time period).

to 1.3 males per female (± 0.1 , $n = 73$) in mid-season, and 0.6 males per female (± 0.1 , $n = 83$) late in the season (Kruskal-Wallis test, $p < 0.0001$).

Seasonal Patterns of Mating Behavior

Seasonal changes in operational sex ratio were associated with marked changes in mating behavior within both *Photinus* species. Early in the flight season there were as many as six males actively engaged in dialogue with a single female. The first male to make physical contact with a female generally ended up as her mate, although early in the season copulating pairs were frequently attended by later-arriving, extraneous males. During stage I, these extra males harassed copulating pairs, climbing over them and repeatedly trying to insert their genitalia between the male and the female; occasionally extra males knocked copulating pairs from their perches. Extra males sometimes remained dorsally mounted after the copulating pairs switched to stage II, but generally departed within 15 min after the start of stage II. Thirteen *P. marginellus* matings were observed that involved extraneous males in contact with a copulating pair, but no successful takeovers by extraneous males were seen.

Early in the flight season females often attracted multiple males, but later in the flight season the situation was reversed with either males or artificial male flashes eliciting simultaneous flash responses from multiple females widely scattered across the mating arena. For *P. marginellus*, there was an increase in the overall response rate of females (proportion of all males' flashes to which a female responded) as local sex ratios became more female-biased (Pearson correlation between female response rate and local sex ratio = -0.29 , $n = 73$, $p = 0.01$). Late in the season we observed several instances of single *P. marginellus* males engaged in active flash dialogues with two or more females that responded synchronously. For the three cases in which detailed observations were made, males preferentially oriented toward and eventually ended up mating with the most responsive female, while the remaining females did not mate. The mean number of female responses per male flash for those late-season females that mated was $45.7\% \pm 14$, compared to $30.6\% \pm 13$ for non-mating females (paired t-test, $n = 3$, $p = 0.02$).

P. marginellus copulatory behavior also changed over the course of the flight season, apparently associated with local sex ratios. Although not many late-season matings were observed because of the paucity of males, an unusually high proportion of these matings (4 out of 14 observed matings) were characterized by repeated bouts of stage I, with copulations never proceeding to stage II. The first stage of copulation increased significantly in duration from early-season (mean duration = 75 sec \pm 12, n = 37) to late-season (mean duration = 283 sec \pm 112, n = 14: Mann-Whitney U test, p = 0.008). Conversely, the duration of stage II decreased significantly from early-season matings (mean stage II duration = 94 min \pm 6, n = 49) to late-season matings (mean duration = 10 min \pm 10, n = 4: Mann-Whitney U test, p = 0.003).

DISCUSSION

In *Photinus aquilonius* and *P. marginellus* both males and females mated repeatedly over the course of their breeding season. In both species males appear to interact primarily through scramble competition for access to stationary females, with the first male to arrive at a female becoming her mate. Direct male-male aggressive interactions did occur early in the flight season of each species when several males reached the female within a short time period. Females appear to give flash responses differentially to particular males, as indicated by higher female response rates to those males that eventually mated relative to the response rates of the same females to non-mating males. The basis of this female selective response to particular males is not known, but higher rates of female response are likely to enhance a male's probability of successfully mating by enabling him to orient toward the female more accurately, and thus reach her more quickly.

The mating systems of *P. marginellus* and *P. aquilonius* are quite distinct from the monogamous mating system reported for *Photinus collustrans*, which was studied in detail by Wing (1984). Associated with their promiscuous mating systems, the *Photinus* species studied here exhibited interesting differences in copulatory behavior. The present study describes two posturally distinct stages of copulation in these *Photinus* species, similar to two copulatory phases noted for the European lampyrid, *Luciola lusitanica* (Papi 1969). Nearly all copulations observed in *P. marginellus* and

P. aquilonius included both copulatory stages, but in *P. collustrans* copulations usually consist only of the dorsal mount of stage I, with behavior analogous to stage II occurring only in the presence of competing males (Wing 1984). Both *P. marginellus* and *P. aquilonius* exhibited fairly long copulation durations (> 1 h), while in *P. collustrans* copulations last only about 1 min. Copulation durations ranging from 1–9 hours have also been noted for *P. macdermotti*, a lampyrid species for which there is some evidence of multiple-mating by females (Wing 1985).

Wing (1985) suggested two possible reasons for prolonged copulation durations in multiply-mating *Photinus*. Relative to *P. collustrans*, the male reproductive systems of *P. macdermotti*, *P. marginellus*, and *P. aquilonius* all have additional accessory glands, including a prominent pair of spiral glands (Wing 1985, S. Lewis unpublished data). Longer copulations may be required to transfer the complex ejaculates produced by males of these multiply-mating species. For *P. macdermotti*, Wing (1985) reported histological evidence that the first half-hour of copulation involved transfer of accessory substances, with sperm transfer apparently occurring later. The relatively long duration of copulation in multiply-mating *Photinus* species may also represent copulatory mate guarding, preventing females from remating with other males. This mate-guarding interpretation of prolonged copulation is further supported by the observation in the present study of reduced copulation duration in *P. marginellus* late-season matings. Under the female-biased sex ratios characteristic of late flight seasons, mate-guarding may not be worth the time invested since there are many additional mating opportunities. McLain (1980) noted a similar effect of sex ratio in the laboratory, with female-biased sex ratios resulting in reduced copulation durations in the Southern stink bug, *Nezara viridula*.

The repeated insertion-withdrawal of the male's aedeagus observed in both *P. aquilonius* and *P. marginellus* during stage I copulation has not been reported previously, and its function is currently unknown. A similar behavior has been described in the damselfly *Ischnura elegans* (Miller 1987), and was found to be associated with removal of sperm stored in the female's bursa from previous matings. As in other insect species with multiply-mating females (Parker 1970; Ridley 1989), sperm competition among

mating males for access to fertilizations is likely to be important in these *Photinus* species. Although the timing of oviposition in the field is not known for these *Photinus* species, females likely mate several times before ovipositing; laboratory observations indicate that females oviposit at irregular intervals between matings (*P. aquilonius* females laid several batches of about 10 eggs over a 20 day period, S. Lewis unpublished data).

Operational sex ratios changed markedly over the flight season for both of these *Photinus* species, with an early peak of male abundance followed by a steady decline in the number of active males. To some extent, similar seasonal patterns may be expected in many insects exhibiting protandry, or early emergence of males relative to females (Wiklund and Fagerstrom 1977; Thornhill and Alcock 1983). Earlier male emergence times have been suggested for *Photinus knulli* (Cicero 1983), and may be responsible for the male-biased sex ratios early in the flight seasons of the two *Photinus* species studied here. Another factor likely to alter *Photinus* operational sex ratio is sex-biased predation occurring throughout the flight season, with male *Photinus* being more heavily preyed upon by a variety of spider predators (Lloyd 1973), as well as by predatory *Photuris* females fatales (Lloyd 1965). Our observations of orb-weaving spiders' apparent ability to induce light flashes from dead or dying *Photinus* males bear further investigation. This intriguing phenomenon may represent a hijacking of the *Photinus* male's signalling system to act as a decoy attracting additional *Photinus* males or other potential prey.

Several aspects of *Photinus* mating behavior appear to change seasonally in concert with operational sex ratios. Early in the season, several males competed for access to each female, and females show low overall response rates to males. In contrast, later in the season with female-biased sex ratios there may be competition among females to attract males, with several females responding synchronously to a single male's flashes. Later in the flight season females appeared more responsive, and males selected the most responsive females as mates. A similar seasonal reversal of breeding sex ratio has been noted for another lampyrid, *Photuris versicolor*, and appears to be associated with a change in the female response rate to simulated homospecific vs. heterospecific flashes in this predatory species (J. Copeland and A. D. Carlson,

personal communication). Such predictable seasonal changes in operational sex ratios may be important within many insect populations in determining not only patterns of mate choice and mate competition, but also the relative intensity of sexual selection acting on males and females.

SUMMARY

This research describes the reproductive behavior of two common New England lampyrid species, *Photinus aquilonius* and *P. marginellus*. Fireflies are ideally suited for behavioral studies because their visually-oriented flash communication systems make their mating behavior extremely accessible. The *Photinus* species studied exhibit promiscuous mating systems, with both males and females mating multiply. Mating behavior appears to be affected by predictable seasonal shifts in operational sex ratios, such that male-male competition and female choice prevail early in the flight season, while interfemale competition and male selectivity may predominate later in the season. Although sexual selection theory has previously ignored such demographic effects, this research suggests that breeding sex ratios may strongly affect mating behavior and mating success within these *Photinus* species.

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LITERATURE CITED

- BUCK, J. AND E. BUCK
1972. Photic signalling in the firefly *Photinus greeni*. Biol. Bull. **142**: 192-205.
- CARLSON, A. D. AND J. COPELAND
1988. Flash competition in *Photinus macdermotti* fireflies. Behav. Ecol. Sociobiol. **22**: 271-276.
- CICERO, J. M.
1983. Lek assembly and flash synchrony in the Arizona firefly *Photinus knulli* Green (Coleoptera: Lampyridae). Coleopt. Bull. **37**: 318-342.

- EMLEN, S. T. AND L. W. ORING
1977. Ecology, sexual selection, and the evolution of mating systems. *Science* **197**: 215–223.
- LLOYD, J. E.
1965. Aggressive mimicry in *Photuris*: firefly femmes fatales. *Science* **149**: 653–654.
1966. Studies on the flash communication system in *Photinus* fireflies. *Univ. Mich. Misc. Publ.* **130**: 1–95.
1973. Firefly parasites and predators. *Coleopt. Bull.* **27**: 91–106.
- MCCLAIN, D. K.
1980. Female choice and adaptive significance of prolonged copulation in *Nezara viridula* (Hemiptera: Pentatomidae). *Psyche* **87**: 325–336.
- MILLER, P. L.
1987. An examination of the prolonged copulations of *Ischnura elegans* (van der Linden) (Zygoptera: Coenagrionidae). *Odonatologica* **16**(1): 37–56.
- PAPI, F.
1969. Light emission, sex attraction and male flash dialogues in a firefly, *Luciola lusitanica* (Charp.). *Monitore Zool. Ital.* **3**: 135–184.
- PARKER, G. A.
1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**: 525–567.
- RIDLEY, M.
1989. The incidence of sperm displacement in insects: four conjectures, one corroboration. *Biol. J. Linn. Soc.* **38**: 349–367.
- THORNHILL, R. AND J. ALCOCK
1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.
- WIKLUND, C. AND T. FAGERSTROM
1977. Why do males emerge before females? A hypothesis to explain protandry in butterflies. *Oecologia* **31**: 153–158.
- WING, S. R.
1984. Female monogamy and male competition in *Photinus collustrans* (Coleoptera: Lampyridae). *Psyche* **91**: 153–160.
1985. Prolonged copulation in *Photinus macdermotti* with comparative notes on *Photinus collustrans* (Coleoptera: Lampyridae). *Florida Entomol.* **68**: 627–634.



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